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IMMUNOLOGICAL DIFFERENCES BETWEEN STRAINS OF POLIOMYELITIC VIRUS.*

F. M. BURNET AND JEAN MACNAMARA.

From the Walter and Eliza Hall Institute, Melbourne.

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ALL who have worked with experimental poliomyelitis in monkeys are agreed that an attack of the disease from which the animal recovers leaves an almost absolute degree of immunity. Even if the infection shows itself only by excitability and transient ataxia without paralysis the monkey is subsequently resistant even to intracerebral inoculation with a highly active virus. This has necessarily led to the view that while different strains of the poliomyelitis virus may show great differences in the intensity of their action on the central nervous system, yet they are immunologically identical. One might compare the different strains of virus with a series of diphtheria toxins inactivated to various degrees by treatment with formaldehyde. All retain the power to provoke a qualitatively identical immunity, but their pathogenic activity ranges from the maximal to complete innocuity.

In the course of work on the neutralizing power of convalescent human sera (Burnet and Macnamara, 1929) several exceptions to this rule of complete subsequent immunity were encountered. The experiments in question were concerned with the response of monkeys, which had been previously infected and paralysed, to a second intracerebral injection of a poliomyelitic virus of different origin. A few experiments on the neutralizing power of convalescent monkey serum for two distinct viruses were also carried out. The two viruses used were those described in our previous communication—a local virus of moderate activity derived from a child dying in the Children's Hospital, Melbourne, in February, 1928, and the highly virulent " mixed virus " strain from the Rockefeller Institute which we received through the courtesy of Dr. Flexner.

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The experiments have had to remain incomplete, but the trend of the results obtained is sufficiently definite to warrant a report. Brief protocols of these experiments may be grouped under three headings:

1. FAILURE OF A PREVIOUS INFECTION WITH LOCAL VIRUS TO PROTECT AGAINST ROCKEFELLER VIRUS.

Monkey 16.—29.1.29. Local virus and convalescent serum were injected intracerebrally. The animal showed no symptoms, and on 6.3.29 was given 0·5 c.c. of local virus alone by the same route. Paralysis of legs was evident on 14.3.29 and became complete. The arms and upper part of the body were unaffected, but almost complete paralysis of both legs with contractions of the hips in flexion remained permanent. On 18.7.29, 0·5 c.c. of a Rockefeller virus filtrate was injected intracerebrally. Five days later the monkey appeared to be sick and its movements were tremulous. This condition lasted till 28.7.29, when the animal became rapidly paralysed and prostrate. The cord was removed, and an emulsion injected intracerebrally into Monkey 56 on 1.10.29. On 7.10.29 this monkey became paralysed, with the typical complete prostration produced by the Rockefeller virus.

Monkey 58.—On 29.1.30 a mixture of local virus and immune human serum was injected, no symptoms being produced. The injection on 18.2.30 of 0·5 c.c. of local virus suspension was followed on 27.2.30 by typical paralysis, first in the right leg, then involving the other leg and right arm. All movements were very tremulous. The condition gradually improved, and by 3.7.30 both limbs on the left side were fairly normal, the right arm was weak, and there was contraction of the right ankle in dorsiflexion and general weakness of this limb. Another injection of local virus, 0·5 c.c., was made intracerebrally on this date without any resulting symptoms. The same suspension injected into Monkey 59 resulted in typical paralysis 12 days later.

On 5.8.30, 0·5 c.c. of a Rockefeller virus filtrate was injected. Five days later there was typical paralysis, with complete prostration and death on 11.8.30.

Monkey 64.—Local virus (0·5 c.c. of suspension) was injected intracerebrally on 5.8.30. On 13.8.30 paralysis of both legs was evident, becoming complete on the following day. There was no involvement of the upper half of the body. The legs remained almost completely paralysed, and by 3.9.30 contraction of the hips in flexion had occurred. On this date a few c.c. of blood were removed and the animal given 0·5 c.c. of a Rockefeller virus suspension intracerebrally. On 6.9.30 the monkey was abnormally excitable, and for the next 4 days was very tremulous in the movements of its arms. The condition was similar to that shown by Monkey 16, but in this case there was no increase in paralysis, and after 11.9.30 the animal appeared normal except for the old paralysis of its legs. The same suspension injected into a control monkey, 65, resulted in the usual paralysis on the 4th day going on to complete prostration within 24 hours.

Each of these three animals was presumably immune to the local virus after the first infection, but all showed some reaction to the second injection of Rockefeller virus. In one there was an apparently unmodified acute infection,

in another the onset of widespread paralysis was preceded by a period of five days of indefinite symptoms, and in the last the symptoms after the usual incubation period indicated an abortive infection without increase in the degree of paralysis.

2. THE NEUTRALIZING POWER OF SERUM FROM A MONKEY PREVIOUSLY PARALYSED BY LOCAL VIRUS.

Serum from Monkey 64 taken just before the second intracerebral injection was made, was tested for its power to inactivate the two viruses according to the usual technique. The results given in Table I show that in a dose of 0·5 c.c. the serum inactivates a local virus suspension but does not modify the infectivity of a Rockefeller virus filtrate.

TABLE I.

Monkey.	Virus.	Serum of Monkey 64, 3.9.30.	Result.
66	Rockefeller suspension	0·1 c.c.	Paralysed 4th day. Dead on 6th.
71	Rockefeller filtrate	0·5 c.c.	Paralysed 4th day. Dead on 5th.
68	Local suspension	0·1 c.c.	Paralysed 11th day. Dead on 12th.
73	Local suspension	0·5 c.c.	Survived without symptoms.

3. INFECTION BY A LOCAL VIRUS IN A MONKEY PREVIOUSLY PARALYSED BY PARTIALLY NEUTRALIZED ROCKEFELLER VIRUS.

Monkey 51.—On 8.8.29 received a mixture of Rockefeller virus filtrate and 0·1 c.c. of Victorian human immune serum (see previous paper, Table VIII). The monkey appeared quiet on the 7th and 8th days after inoculation, and on the 9th was holding the right arm in semiflexed position and not using it. It was very unsteady on its feet, tending to topple forwards. The weakness of gait improved rapidly, but partial paralysis and contracture of the right arm persisted. On 1.10.29, 0·5 c.c. local virus suspension was injected intracerebrally. The monkey was tremulous and weak in the legs on 7.10.29, and completely paralysed the following day.

Several attempts were made to obtain other survivors who had been paralysed with Rockefeller virus by injecting partially neutralized mixtures of virus and serum. Beyond a lengthening of the incubation period, however, it was found impossible to modify the infection short of its complete elimination. This agrees with the results of Fairbrother and Hurst (1930), who used the same virus for a large number of experiments and observed only one instance in which a monkey was paralysed but recovered. In this case the virus was administered together with serum from an immunized horse.

DISCUSSION.

The results of these experiments show clearly that paralysis by poliomyelitis is not necessarily indicative of complete resistance to subsequent infection, provided that the second infection is with a virus of greater virulence or different type from the first. The question arises as to whether the differences between viruses are sufficient to justify differentiation into immunological types, corresponding, for example, to the A and O types of foot and mouth disease. The evidence given above shows conclusively that infection and paralysis with our Australian virus usually does not protect against the American virus. Monkey 58 shows this particularly clearly. After being paralysed by local virus a subsequent injection of the same virus was without effect. A normal monkey used as a control showed typical poliomyelitis. The animal was therefore immune to the local virus, but the next injection with a Rockefeller virus filtrate showed that no resistance to this virus had been acquired. There was not even any increase in the incubation period of the disease.

Even in the one case (Monkey 64) that showed no increase in paralysis, there was fairly clear evidence that infection by the Rockefeller virus occurred at the usual time after injection, and the serum of this monkey had power to neutralize *in vitro* only the Victorian virus. Unfortunately the very high virulence of the Rockefeller virus, which in our hands has always caused paralysis on the 4th or 5th day with complete prostration and death within one or two days, has prevented attempts to test its power to immunize against the local virus. The one monkey available (Monkey 51) had only a moderate degree of paralysis after receiving a mixture of Rockefeller virus and a dose of human immune serum which must have been very close to the neutralizing dose. The time of onset of the paresis after inoculation and its general character make it almost certain that the monkey had a genuine infection with the Rockefeller virus attenuated by contact with immune serum. On subsequent testing it showed no resistance to the local virus.

Although the findings are compatible with the view that two immunologically distinct strains of virus are concerned, we feel that a more conservative interpretation is advisable unless it can be shown that monkeys *severely* paralysed by the Rockefeller virus are still susceptible to the Australian one. The work of Stewart and Rhoads (1929) has shown that there are definite degrees in the immunity that can be induced in monkeys by intradermal or subcutaneous inoculation of poliomyelitis virus. After a prolonged course of intradermal injections monkeys were found to be resistant to intracerebral inoculation of a moderately active virus (M.A.), but were not completely resistant to inoculation by the same route with a stronger virus (" Aycock "). Further, one monkey which had been proved resistant to " Aycock " was found still to be susceptible to the highly virulent " M.V.," which is the mixed Rockefeller strain used in our own experiments. Monkeys inoculated subcutaneously were less solidly immune. They resisted injections of "M.A.," but showed typical poliomyelitis after inoculation with " Aycock " instead of the abortive symptoms produced in the intradermally immunized animals. But all but one of the monkeys immunized by either method developed antibodies in their sera which were capable of neutralizing *in vitro* even the highly active " M.V."

In many respects these results are analogous to those reported above in showing the existence of degrees of immunity, but there is the important distinction that the monkeys used in our experiments had been frankly paralysed by the first attack of poliomyelitis. It has been the general opinion of experimenters that all monkeys which showed any definite poliomyelitic symptoms after inoculation, even a transient tremor, or ataxia were thereafter immune to inoculation with a virulent strain of virus. Flexner and Amoss (1924), for example, described an attenuated strain which rarely produced permanent paralysis, but which gave a solid immunity against an active virus.

It seems justifiable, therefore, to interpret our results as indicating definite minor immunological differences between the two strains used. The most rigid test of immunity is the intracerebral injection and, judged by this criterion, there is a relatively sharp difference between the two viruses. This is substantiated by the difference in neutralizing power for the two viruses shown by the serum of Monkey 64. On the other hand, there is definite evidence that the two are not completely distinct, since Monkey 64 was not further paralysed by the invariably fatal " M.V." virus, and since pooled human convalescent sera are approximately equally active against both strains (Burnet and Macnamara, 1929). It is of interest to note, however, that some recent tests with serum from Sydney convalescents has failed to show this parallelism, only the Victorian virus being neutralized. The number of experiments (two with each virus) is too small for the observation to be considered significant.

CONCLUSIONS.

A poliomyelitic virus derived from a child dying in Melbourne has shown distinct immunological differences from the Rockefeller Institute " mixed virus " strain both in cross-immunity experiments and by neutralization tests *in vitro*.

Three instances are described of monkeys which contracted a typical fatal infection after injection of the heterologous virus, despite the fact that some weeks previously they had suffered a typical attack of experimental poliomyelitis.

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